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Tenascins, a growing family of extracellular matrix proteins

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Abstract. The tenascins are a family of large multimeric extracellular matrix proteins consisting of repeated structural modules including heptad repeats, epidermal growth factor (EGF)-like repeats, fibronectin type III repeats, and a globular domain shared with the fibrinogens. The tenascins are believed to be involved in the morphogenesis of many organs and tissues. To date three members of the tenascin family have been described, tenascin-C, tenascin-R, and tenascin-X. Tenascin-R seems to be specific for the central and peripheral nervous system, tenascin-X is most prominent in skeletal and heart muscle, while tenascin-C is present in a large number of developing tissues including the nervous system, but is absent in skeletal and heart muscles. Tenascin-C was the original tenascin discovered, partly because of its overexpression in tumors. Inferring from cell biological studies, it has been proposed that tenascin-C is an adhesion-modulating protein.
Key words. Tenascin; extracellular matrix; cell adhesion; fibronectin; multidomain structure; heparin-binding; morphogenesis.

Introduction

The tenascins are a family of extracellular matrix proteins for which the use of a coherent nomenclature was recently proposed, namely tenascin-C, tenascin-R and tenascin-X, for the three members currently known[10,28]. If we include in this list other proteins containing tenascin-type epidermal growth factor (EGF)-like repeats, three more members of this extended family can be recognized, namely the two *Drosophila* proteins Ten^a and Ten^m and the presumptive *C. elegans* protein encoded by its recently discovered *ten*^{m} gene. A compilation of data concerning all members, including their original discoveries, their previous names and the accession numbers required for recovering the corresponding cDNA or gene sequence is shown in the table. Diagrams depicting the structural models of the extended tenascin family members are shown in figure 1.

Tenascin-C structure and splicing variants

Tenascin-C is a disulfide-linked hexameric extracellular matrix protein with subunit molecular weights in the range of 190–300 kDa depending on the species analyzed. Different subunits are generated by alternative splicing of one common primary transcript. The electronmicrograph and the model shown in figure 2 reveal the domain organization of chicken tenascin-C[92]. The most prominent structural domains, which are arranged like beads on a string, are: heptad repeats, tenascin-type EGF-like repeats, fibronectin type III repeats and a fibrinogen domain. The heptad repeats enable three tenascin-C subunits to trimerize in a triple coiled coil region which is stabilized by adjacent disulfide bridges. The EGF-like repeats are special in the sense that they are the shortest EGF-like repeats found so far in any proteins.

Therefore, if a tenascin-type EGF-like repeat is modelled after the known nuclear magnetic resonance (NMR) structure of human EGF, two of the loops between the three disulfide bridges contained in each repeat are much shorter[15]. When the fibronectin type III repeats in tenascin-Cs from different species are aligned according to their highest similarity, it becomes clear that human tenascin-C[66,91,93] contains more extra repeats subject to alternative splicing than has been described for the chicken[42,92,103] or mouse[25,81,111] counterparts. The higher number of extra repeats is due to a recent duplication within the tenascin-C gene[38]. In order to simplify the comparison between tenascin-Cs of different species, the nomenclature presented in figure 3 has been proposed[3,17]. The constant repeats are numbered whereas the extra repeats are letter coded. Each tenascin-C contains eight constant repeats that are numbered from 1 to 8, as well as various numbers and types of extra repeats that are shaded and letter coded. Repeat A has been found once in chicken, three times in mouse and four times in human tenascin-C. Repeat C was originally found to exist only in human tenascin-C but was recently identified in certain chicken[103] and mouse[25] tenascin-C variants as well. Repeat AD1 was also first identified in human tenascin-C[93], but we recently discovered its presence in chicken tenascin-C, where we also found a novel repeat termed AD2 which has not been described in any other tenascin-C[103]. The model of chicken tenascin-C presented in figure 3 most likely includes a complete set of all possible fibronectin type III repeats, since sequencing of the genomic region

Table. Independent discoveries of tenascins and their primary sequences.

Type of tenascin	Other names	Sequence information/ accession number
Human tenascin-C	glioma mesenchymal extracellular matrix antigen – GMEM[9] hexabrachion[30]; brachionectin[31] neuronectin[35]	ddX56160[91] M96686[93] M55618[66]
Mouse tenascin-C	tenascin[2] J1[49], J1-200/220[33]	X56304[113]; D90343[81]
Rat tenascin-C	tenascin	U09361; U09400; U09401[51] U15550
Pig tenascin-C	tenascin	X61599[67]
Chicken tenascin-C	myotendinous antigen[12,13] cytotactin[37]	M23121[92]; X73833[103] J04519[42]
Newt tenascin-C	tenascin	M76615[70]
Frog tenascin-C	tenascin	X68620[104]
Fish tenascin-C	none	U14940[1]
Rat tenascin-R	J1[49]; J1-160/180[75]; janusin[85]	Z18630[34]
Chicken tenascin-R	restrictin[78]	X64649[68]
Human tenascin-X	gene X[63] tenascin-like gene[58,59]	M25813[63]; X71923-X71938[10] X60189[58]; X62489[59]
Mouse tenascin-X	none	X73959[60]
Drosphila ten^{a}	none	X68794[6]
Drosophila ten^{m}	*odd Oz*[52]	X73154[7]
C. elegans ten^{m}	gene R13F6.4[113]	U00046[113]

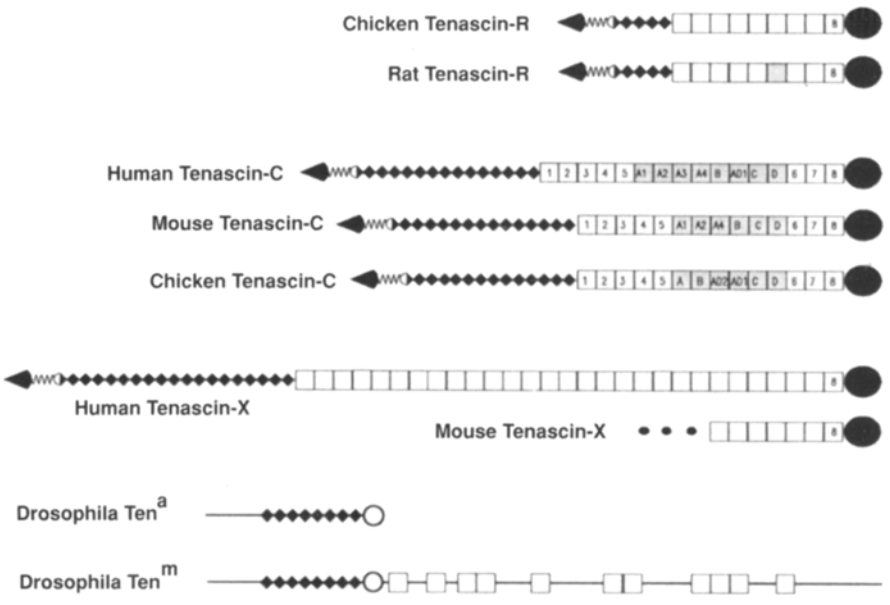

Figure 1. Structural models of the members of the tenascin family. The drawings are based on the published primary structures of chicken tenascin-R[65], rat tenascin-R[32], human tenascin-C[86,63,88], mouse tenascin-C[105,77], chicken tenascin-C[40,87,98], human tenascin-X[8], mouse tenascin-X[57], *Dropophila ten*^{a}[4] and *Drosophila ten*^{m}[5]. The same symbols are used to designate the various domains as described in figure 2*B*. The *Drosophila* proteins contain an additional cystein-rich domain drawn as an open circle. The exact number of fibronectin type III repeats present in human tenascin-X is not known yet, but since so far 29 repeates have been found to be present in the tenascin-X gene[8], this minimal number of 29 repeats is included in the model. The structure of mouse tenascin-X is incomplete and the missing part is indicated by dots.

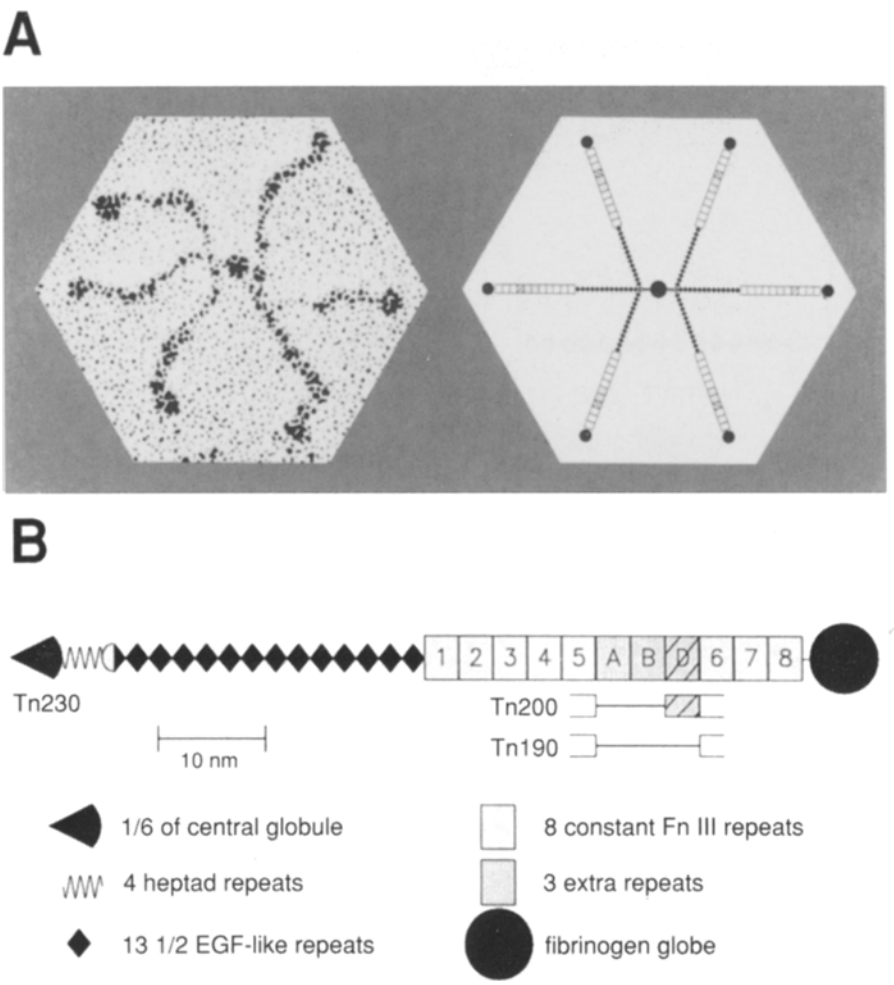

Figure 2. Structure of chicken tenascin-C.
A An electromicrograph of a tenascin-C molecule after rotary shadowing is shown to the left of a structural model of the hexameric molecule.
B The stuctural domains building up one tenascin-C subunit are shown. Alternative splicing of one common tenascin-C transcript leads to the generation of the three major splicing variants observed in the chicken of 230 kDa (Tn230), 200 kDa (Tn200), or 190 kDa (Tn190). The three fibronectin type III repeats subject to alternative splicing (extra repeats) are shaded.

spanning repeat A to D did not reveal the presence of any further exons[103]. For the other tenascin-Cs, the models may not represent the final picture. Various other repeats can be expected to exist in the different tenascin-C species.

Tenascin-C tissue distribution and cellular source

There exists a wealth of information concerning the tissue distribution of tenascin-C. Its pattern of expression is extremely variable depending on the developmental stage of the organism analyzed, and its expression changes dramatically under many different pathological conditions. During normal embryogenesis, tenascin-C is especially prominent in the developing central nervous system, in the matrix lining the pathways of migratory cells, in mesenchyme at sites of mesenchymal-epithelial interactions, and in developing connective tissues (for reviews see refs 26, 29).

The cellular source of tenascin-C transcripts, and thus most likely the tenascin-C protein, has in many instances been investigated using in situ hybridization. In the central nervous system the cellular source of tenascin-C seems to be the glial cells[97,99,101,102], the satellite cells surrounding the outgrowing motoraxons of the peripheral nervous system[110] and neutral crest cells[102].

Developing skeletal connective tissue cells synthesize tenascin-C[56,72,103] as well as the cells of soft connective tissues[12,110]. At sites of mesenchymal-epithelial interactions, the cellular source of tenascin-C was in many cases believed to be the connective tissue cells surrounding the developing epithelium. This assumption was based on the presence of immunostaining in the mesenchyme but not the epithelium. However, this view may need to be revised at least to some extent, since in the case of the lung[48,77] and feather buds[98] it was shown by in situ hybridization that tenascin-C mRNA is de-

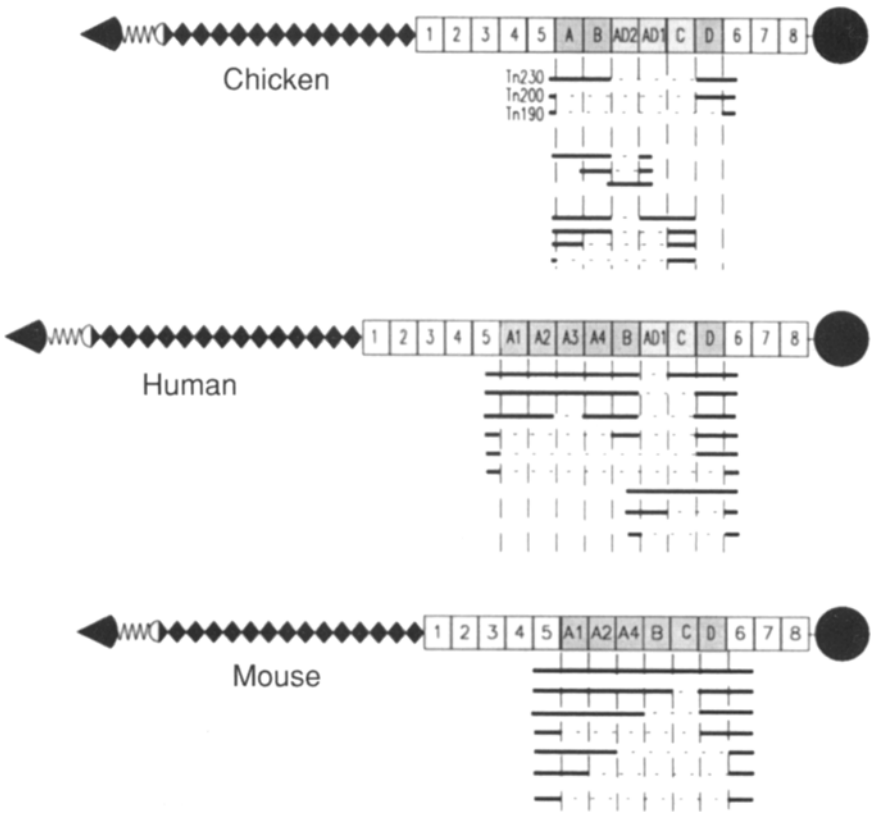

Figure 3. Comparison between chicken, human, and mouse tenascin-C. The constant fibronectin type III repeats are numbered, whereas the alternatively spliced extra repeats are shaded and letter coded. Below each subunit the reported splicing variants are shown.

tected firstly in the epithelium and only later in the mesenchymal cells. Since in these cases the epithelial tenascin-C also accumulates in the mesenchyme[48,98], most of the immunofluorescence data concerning the site of production of tenascin-C must be interpreted carefully. Recently, it was shown that several carcinoma cell lines were able to secrete tenascin-C in tissue culture[46,53] and that in sections of normal breast tissue, the ductal epithelial cells contained tenascin-C transcripts[53]. Furthermore, in all breast carcinomas studied, tenascin-C transcripts were not only found in the tumor stroma where the tenascin-C protein accumulates but also in the tumor epithelium, particularly at the periphery of the tumor lobules[53]. Clearly the idea that fibroblasts and not epithelial cells are the primary producers of tenascin-C has now been disproven.

Apart from the overall tissue distribution of tenascin-C, which develops in a complex temporal and spatial pattern, we can also observe a differential expression of tenascin-C splicing variants in different tissues and at different stages of development[21,25,45,56,77,103] or under different pathological conditions[8,71,87,90]. This intricate regulation of the expression of tenascin-C splicing variants leads to the interesting question of whether the numerous extra repeats convey specific functions to tenascin-C.

In humans there are some tissues in which tenascin-C is present in the adult[65,69]. These include perichondrium and periosteum, some but not all smooth muscles, certain parts of the nervous system, hematopoetic organs, gut and skin. In the skin, the distribution of tenascin-C changes from a patchy distribution within the dermal papillae to an intense staining deep into the dermis during wound healing[23,55,112], and hyperproliferative skin diseases such as psoriasis[86] and scleroderma[50] as well as in bullous deseases[87]. In normal liver, low amounts of tenascin-C can be detected. However, its expression is upregulated in many liver diseases[105,106]. Most strikingly, tenascin-C expression is upregulated in most tumors analyzed including gliomas, melanomas, and most types of carcinomas (for a recent review see ref. 17). It was reported that in melanoma patients with a high burden of metastases, tenascin-C levels were elevated in the patient sera[39]. Furthermore, it was suggested that the determination of tenascin-C levels in patient sera could be used as a diagnostic tumor marker, since carcinoma patients were also found to have elevated levels of tenascin-C[47,80,107]. In our own studies, we could not confirm the general usefulness of serum measurements of tenascin-C as a diagnostic tool for tumor patients. We found the highest levels of tenascin-C in sera of patients with acute sepsis and not

in tumor patients. Furthermore we found a correlation of tenascin-C levels with the acute phase reactant C-reactive protein[88,89]. It is therefore possible that tenascin-C is induced as an acute phase protein in the liver through the action of inflammatory mediators such as interleukins. Indeed, many growth factors and cytokines have been shown to induce the synthesis of tenascin-C in vitro. TGF-β[73], bFGF[62,101], IL-1[61], IL-4 and TNFα[79] have all been reported to upregulate tenascin-C synthesis. Interestingly, in Swiss 3T3 fibroblasts TGF-β1 and bFGF were shown to induce specifically small or large tenascin-C variants, respectively[101]. Furthermore, it was shown that upon stimulation by various growth factors and cytokines, different cell types respond by expressing variable amounts of tenascin-C[79].

Tenascin-C function?

The common test for the function of any protein, namely knocking out its gene in transgenic mice, has been performed by Saga et al.[82]. The title of this report says everything: 'Mice develop normally without tenascin'. Clearly this approach has not yet yielded a quick answer about the function of tenascin-C. It remains to be determined whether these mice have a more subtle phenotype yet to be discovered, which could point towards a possible function of tenascin-C. Alternatively, these mice will have to be investigated for mechanisms of compensation for the loss of tenascin-C (for further discussions on the absence of a phenotype of the tenascin-C-less mice see refs 18, 27, 100).

Because of the lack of in vivo data concerning tenascin-C function, our ideas about possible functions to tenascin-C are inferred from its tissue distribution and from cell and organ culture experiments. Originally, it was found that tenascin-C is in general a poor adhesion substrate for most cells in culture, and if the cells *are* able to adhere to the tenascin-C they often do not spread out (for reviews concerning this aspect see refs 16, 17, 83). The integrins $\alpha 2\beta 1$ and $\alpha v\beta 3$ have been proposed as the cellular tenascin-C receptors of endothelial cells[43,94] as well as various αv-containing integrins on glioma and carcinoma cell lines[76]. Adhesion through the αv-containing integrins seems to involve the tripeptide RGD present in the third fibronectin type III repeat of chicken and human, but not of mouse tenascin-C[43,76,94]. It appears, however, that the RGD adhesion site of tenascin-C may be a cryptic site, which is covered up by the adjacent second fibronectin type III repeat[43]. Based on antibody inhibition data, more important cell adhesion sites of tenascin-C appear to be located in the three most C-terminal fibronectin type III repeats[54,92] and the adjacent fibrinogen globe[43]. In addition to integrins, tenascin-C also seems to bind to the heparansulfate side chains of syndecan[84]. This binding activity may be related to the heparin-binding activity of tenascin-C which is located in the C-terminal fibrinogen globe[3,14]. Tenascin-C was shown to promote neurite outgrowth, and this activity could be blocked by either the addition of anti-β1 antiserum or heparin[109], suggesting the cooperation of two types of receptors in the process of neurite growth. Further candidates for neuronal tenascin-C receptors are the cell adhesion molecule F11/contactin[117] and the receptor tyrosine phosphatase β[4], both of which have been shown to bind to tenascin-C.

In contrast to the adhesion of growth cones, neuronal cell bodies adhere poorly to tenascin-C substrates[32,108]. Furthermore, glial cells avoid migration out of ganglia plated on tenascin-C[109]. Tenascin-C often showed anti-adhesive effects on cells. Cell adhesion and spreading of fibroblasts on fibronectin can be inhibited by the addition of tenascin-C to the culture medium[20]. Similarly, the addition of tenascin-C to endothelial cells inhibits focal contact formation[64] and inhibits epithelial cell adhesion[19,44]. In the case of the endothelial cells[64] and the uterine epithelial cells[44], the extra repeats of tenascin-C were found to be responsible for the anti-adhesive effect, while the anti-adhesive effect observed for fibroblasts seemed to rely on the presence of the EGF-like repeats of tenascin[92]. Very recently, annexin II was identified as a high affinity receptor for the extra repeats of tenascin-C[22]. It remains to be determined whether this interaction leads to their anti-adhesive effects. In slightly more complex organ culture experiments, it was found that anti-tenascin-C antibodies block cerebellar granule cell migration[24,41] and inhibit the branching morphogenesis of fetal lung explants[116]. It is conceivable that some of the effects observed following the addition of tenascin-C to various cell culture model systems could rely on its potential to induce, in conjunction with fibronectin, the expression of collagenase, which interestingly is dependent on the presence of heparansulfate on the responsive cells[96].

Tenascin-R

Tenascin-R was discovered as a component of a mixture of mouse brain glycoproteins termed J1 protein[49], which upon further characterization could be separated into a J1-200/220 fraction representing tenascin-C[33] and a J1-160/180 fraction representing tenascin-R (or janusin)[75]. Independently, chicken tenascin-R, then termed restrictin, was discovered as a brain extracellular matrix ligand of the neural cell adhesion molecule F11/contactin[78]. The binding of chicken tenascin-R to F11/contactin has been shown to depend on the second or third IgG domain of F11/contactin[11]. This may be the same region where tenascin-C has been proposed to bind F11/contactin, namely within the three N-terminal IgG domains[117]. Molecular cloning of the chicken restrictin[68]

and the rat J1-160/180[34] revealed that these two proteins were species homologues. Structurally, tenascin-R consists of the same types of domains as tenascin-C (cf. fig. 1), but in contrast to tenascin-C which forms hexamers, tenascin-R preparations contain trimers, dimers and monomers, but never any hexameric molecules[68,75].

In situ hybridization studies revealed that mouse tenascin-R transcripts were exclusively detected in the central nervous system and were most highly expressed by oligodendrocytes during myelinization, as well as by neurons at early developmental stages during phases of active neurite outgrowth[5,114]. Whereas the tenascin-R expression of oligodendrocytes is down-regulated during later stages of development, neuronal expression of tenascin-R persists in the adult central nervous system. In the developing chicken nervous system, immunohistochemistry revelaed the presence of tenascin-R in axon-rich regions of the cerebellum, the retina and in motor axons of the spinal cord[78]. On Northern blots of chick brain RNA, tenascin-R mRNA is first detectable at embryonic day six, reaches a maximum at embryonic day 16 and remains present at a reduced level in the adult brain[68].

Chicken retinal cells were shown to adhere to a tenascin-R substrate, but they did not grow any neurites[78]. Furthermore, mouse tenascin-R was shown to be a repulsive substrate for neurons, astrocytes and fibroblasts[75]. The repulsion of neurons was shown to be mediated by F3/F11/contactin[74]. In contrast, under certain circumstances tenascin-R appears to accelerate neurite outgrowth of dorsal root ganglion neurons on a mixed substrate with laminin as compared with laminin alone[95].

Tenascin-X

A gene was discovered which overlaps with the opposite strand of the human steroid 21-hydroxylase/complement component C4 gene locus (the major histocompatibility complex (MHC) class III locus), and was termed human gene X[63]. Upon analysis of this gene sequence and the characterization of further exons, it became clear that it is highly homologous to tenascin-C and consists of the same type of domains as tenascin-C[36,58,59,115]. This prompted the unification of the nomenclature for tenascin-like genes and gene X was from then on called tenascin-X[10,28]. From the combined sequence data it could be predicted that human tenascin-X is composed of an NH_2-terminal domain, followed by four heptad repeats, 18.5 tenascin-type EGF-like repeats, at least 29 fibronectin type III domains and a carboxy-terminal fibrinogen domain (cf. fig. 1). Recently, a partial cDNA encoding mouse tenascin-X, consisting of the seven N-terminal fibronectin type III repeats and the fibrinogen globe, was also reported[60].

From Northern blot and RNase protection data of human tissues, it was known that tenascin-X transcripts were ubiquitously expressed, with the highest levels detectable in muscle and testis[10,36]. Antibodies were raised against recombinant fragments of mouse tenascin-X expressed in bacteria[60]. These antibodies recognized a protein with an estimated molecular weight of 500 kDa in heart extract and in the conditioned medium of a renal carcinoma cell line. The subunit size of the tenascin-X protein suggests that it may contain up to 40 fibronectin type III repeats. Immunofluorescence studies of mouse tissues[60] confirmed the prominent expression of tenascin-X in the extracellular matrix surrounding the muscle cells of heart and skeletal muscles. In skin and the developing digestive tract, a reciprocal distribution of tenascin-X and tenascin-C was observed. Furthermore, in all tissues analyzed, tenascin-X antibodies stained blood vessels, explaining the ubiquitous presence of tenascin-X mRNA. In contrast to tenascin-C and tenascin-R, tenascin-X was not detectable in the nervous system.

Little is known about the function of tenascin-X. Using in vitro binding studies, tenascin-X was shown to bind to heparin, but no binding to tenascin-C, fibronectin, laminin or collagens could be detected[60]. Patients with congenital adrenal hyperplasia carry deletions in their 21-hydroxylase gene which overlaps the gene of human tenascin-X. The fact that such deletions never extended into the coding region of tenascin-X led to the assumption that the tenascin-X gene is essential for life[63].

Related invertebrate molecules

It is not yet known whether tenascin homologues consisting of the same domains arranged in the same order as the vertebrate tenascins exist in invertebrates. Hexameric molecules with similar dimensions to tenascins have been isolated from the leech[57] and from the sponge Porifera *Oscarella tuberculata*[40]. Nothing is known yet about the primary structure of these proteins and it remains to be determined whether they are similar to tenascin at the level of their amino acid sequences.

In *Drosphila*, two genes have been isolated that show sequence homology to vertebrate tenascins. These are called *ten*^{a} and *ten*^{m}, respectively[6,7]. Furthermore, a partial sequence of a gene termed *odz* has recently been reported[52], which shows sequence identity with the *ten*^{m} gene. Both Ten^{a} and Ten^{m} contain tenascin-type EGF-like repeats as well as a common cysteine-rich domain not found in vertebrate tenascins. As can be seen in figure 1, Ten^{a} does not contain any additional domains, whereas Ten^{m} harbors fibronectin type III repeats, but no domain homologous to fibrinogen. The fibronectin type III repeats, however, do not resemble the fibronectin type III repeats of the vertebrate tenascins any more than the fibronectin type III repeats of other

molecules. Since Ten^m does not show exactly the same molecular organization as the vertebrate tenascins, and the EGF-like repeats are the only region of the protein with significant homology to the vertebrate tenascins, we cannot claim that Ten^m is an invertebrate homologue of tenascin, but it is the nearest relative known to date. A database search has revealed a *C. elegans* gene, termed R13F6.4[113], whose gene product shows 29% identity to Ten^m over the entire length of 2531 amino acids including the conservation of the Ten^m domain structure. Ten^a is a monomeric protein of 100 kDa molecular weight (S. Baumgartner, unpubl.). The Ten^m protein is a large proteoglycan with a core protein of about 270 kDa[7].

The *Drosophila ten^a* is most highly expressed on the axons of certain neurons, in brain, near muscle attachment sites during embryogenesis, and in the eye during pupal stages[6]. Interestingly, the Ten^m protein is found in seven stripes during the blastoderm stage, and each stripe overlaps with the seven strips of the pair-rule gene even-skipped[7]. During later stages, Ten^m protein is found in heart, lung, eye, muscle attachment sites and on axons.

Ten^m mutants show a phenotype resembling that of odd-paired, another member of the pair-rule class of segmentation genes. Thus, *ten^m* is a new member of the pair-rule gene class and is the first example of a pair-rule gene product potentially acting from outside the cell[7]. The interesting phenotype of *ten^m* mutants raises the question of whether vertebrate tenascins could have similar functions in organizing the segmental body plan.

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